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Fast analysis of glycosaminoglycans by microchip electrophoresis with in situ fluorescent detection using ethidium bromide

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Abstract

We analyzed some glycosaminoglycans and natural and artificial acidic polysaccharides using microchip electrophoresis in the buffer containing ethidium bromide, and found that they were successfully separated and detected within 150 s with comparable sensitivity with that of conventional electrophoresis using cellulose acetate membrane. We applied the technique to the analysis of glycosaminoglycans in pharmaceutical preparations and also in cultured cancer cells. Rapidness and easy operation of the proposed technique are quite useful for routine analysis of glycosaminoglycans.

Keywords: Microchip electrophoresis; Glycosaminoglycan; Ethidium bromide; In situ fluorescent detection

1. Introduction

Microfluidic separation system such as microchip electrophoresis is an emerging attractive technology due to its rapidness, high sensitivity and wide applicability to the analysis of various biological materials. Microchip electrophoresis has been applied to the fast and sensitive analysis of the nucleic acids [1,2], proteins [3], peptides [4], amino acids [5], carbohydrates [6,7], and DNA fragments [8,9]. A few manufacturers have developed microfabricated analytical devices equipped with multi-separation channels enabling high-throughput analysis of genomics and proteomics. Separation performance of microchip electrophoresis is basically similar to that of conventional capillary electrophoresis, but microchip electrophoresis can be performed on a time scale of seconds.

A variety of modern chromatographic techniques such as high-performance liquid chromatography, polyacrylamide gel electrophoresis and capillary electrophoresis have been employed for the analysis of carbohydrates including oligo- and polysaccharides [10]. However, most of the methods require derivatization of carbohydrates with chromophores or fluorophores for sensitive detection prior to the analysis [11]. Furthermore, they should be converted to ions when analyzed by electrophoretic methods.

A group of acidic polysaccharides, glycosaminoglycans (GAGs) such as heparin, heparan sulfate, chondroitin/dermatan sulfates and keratan sulfate, play important roles as major constituents of proteoglycans. Hyaluronic acid, one of the major constituents of tissue matrices, is present as free form and not as a conjugate with protein. GAGs are linear macromolecules composed of repeating disaccharide units of an uronic acid and an aminosugar, and are highly charged because some of the amino and hydroxyl groups are often substituted with sulfate groups [12]. Heterogeneity of GAGs in structure and molecular masses is specific to tissues or species, and plays a key role in various physiological events [13].

GAGs are usually analyzed by the following two methods [14]. (1) Analysis of the oligosaccharides after digestion of the parent GAGs with specific enzymes. After GAGs are digested with lyases or hydrolases, free or unsaturated oligosaccharides thus produced are analyzed by gel electrophoresis [15], high-performance liquid chromatography [16], or capillary electrophoresis [17] after fluorescent

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labeling with 2-aminobenzamide [18], 7-amino-naphthalene-1,3-disulfonic acid (ANDSA) [19], and some other fluorescent compounds. (2) Analysis of native GAGs by electrophoresis. Electrophoresis of GAGs using cellulose acetate membrane is still widely used due to its easy operation and multi-sample-loading ability [20], and allows determination of native GAGs at microgram level, although several hours for electrophoresis and staining steps are required.

In this paper, we propose a rapid method for analyzing native GAGs or acidic polysaccharides by microchip electrophoresis with in situ fluorescent detection in the presence of a fluorescent intercalator reagent. The proposed method shortens the total analysis time in the scale of seconds.

2. Experimental

2.1. Materials

Three preparations of hyaluronan (HA, derived from *Streptococcus zooepidemicus*, rooster comb and pig skin) were obtained from Nacalai Tesque (Nakagyo-ku, Kyoto, Japan), Wako Pure Chemicals Co. (Dosho-machi, Osaka, Japan) and Seikagaku Kogyo (Nihon-bashi, Tokyo, Japan), and have average molecular masses of 1,500,000–2,000,000, 800,000 and 40,000–60,000, respectively. Chondroitin sulfate A (whale cartilage), chondroitin sulfate C (shark cartilage), dermatan sulfate (pig skin) and heparan sulfate (bovine kidney) were obtained from Seikagaku Kogyo. Heparin (pig intestine), low-molecular-weight heparin, fucoidan (*Fucus vesiculosus*) and dextran sulfate were purchased from Sigma–Aldrich, Japan (Chu-o ku, Tokyo, Japan). Colominic acid (*E. coli*) was a generous gift from Dr. Ohta (Marukin-Bio, Uji, Kyoto, Japan). Ethidium bromide and acridine orange were from Sigma–Aldrich, Japan, and Wako Pure Chemicals Co. (Dosho-machi, Osaka, Japan), respectively. Propidium iodide, ethidium homodimer-1 and Hoechst 33258 were from Molecular Probes (Eugen, OR, USA). Pharmaceutical preparations of hyaluronic acid and heparin were donated from Kinki University Nara hospital. Other reagents and solvents were HPLC grade or the highest grade commercially available. Water was purified with a Milli-Q Purification System (Waters, Shinagawa, Tokyo, Japan).

2.2. Apparatus

Microchip electrophoresis was performed using a Hitachi Microchip electrophoresis apparatus (Type SV1100) with an LED detector. The median excitation wavelength of the LED light is preset at 470 nm by the manufacturer and the detection filter for 580 nm or longer wavelengths is installed. The chip made of polymethylmetacrylate (PMMA) has a simple cross channel of 100 μm width and 30 μm depth. The distances from the channel intersection to the sample, sample waste, buffer and buffer waste wells are 5.25, 5.25, 5.75 and 37.5 mm, respectively. The effective length for separation is 30 mm.

2.3. Microchip electrophoresis

Sample loading and analysis procedures were performed according to the recommendation procedure provided by the manufacturer. Buffer solutions were introduced into the microchannels with a syringe. All reservoirs on the microchip were filled with either running buffer or a sample solution prior to the analysis. In the sample-loading step, 300 V was applied to the sample well, and separation was performed by applying the potential of 750 V (130 V at the sample introduction side).

2.4. Cellulose acetate membrane electrophoresis

Cellulose acetate membrane electrophoresis was performed using a SE-33 apparatus (Toyo kagaku). SELECA-V (Advantec Toyo) was employed as cellulose acetate membrane. The membrane was previously immersed in 25% aqueous methanol for a few minutes, and incubated for 30 min in buffer solution for electrophoresis (0.1 M pyridine–0.47 M formic acid, pH 3.0). Samples were spotted at 1.5 cm from the bottom of the membrane with 1.0 cm intervals between each sample spot. Electrophoresis was performed using constant current mode of 0.5 mA/cm for 1 h. After electrophoresis, the membrane was incubated for 10 min in 0.1% Alcian blue/0.1% acetic acid, and then incubated in 0.1% acetic acid for a few minutes to decolorize the background.

2.5. Analysis of glycosaminoglycans (GAGs) from HeLa cells

HeLa cells were cultured in DMEM containing 10% newborn calf sera (NCS) under 5% CO_2 atmosphere at 37 °C. The cells were harvested with a cell scraper, and washed with phosphate buffered saline (PBS, 10 ml) several times. Preparation of GAGs from cells was performed by the similar method as described in the previous report [21]. Briefly, cells (10^8 cells) were suspended in 20 mM phosphate buffer (pH 7.0, 1 ml) and homogenized with a Teflon–glass homogenizer. The homogenate was centrifuged at 8000 g for 10 min at 4 °C, and the supernatant was diluted with 0.5 M Tris–HCl buffer (pH 8.0, 1 ml). The mixture was digested with pronase (2 mg) overnight. After heating the mixture on the boiling water bath for 10 min, the mixture was centrifuged at 8000 × *g*, and the supernatant was collected. A 95% ethanol containing 1.3% potassium acetate and 0.27 mM EDTA (6 ml) was added to the mixture, and kept at 0 °C for 2 h. The precipitate was collected and dissolved in 0.5 M NaOH/1.0 M $NaBH_4$, and the solution was incubated at room temperature for 48 h to release the GAG chains from the peptide. After incubation, GAGs were collected by ethanol precipitation as described above. The precipitate (500 μg) was dissolved in 100 μl of water, and a portion of the solution was analyzed by microchip electrophoresis.

2.6. Digestion of GAGs with chondroitinase ABC

A portion (50 μl) of GAG solution derived from HeLa cells was evaporated to dryness by a centrifugal evaporator (SpeedVac, Servant), and dissolved in 100 mM Tris–HCl buffer (pH 8.0, 50 μl). An aqueous solution of chondroitinase ABC (500 munits, 10 μl) was added. After incubating the mixture overnight at 37 °C, the mixture was kept in a boiling water bath for 5 min. After centrifugation, the supernatant was analyzed by microchip electrophoresis.

3. Results and discussion

3.1. Selection of a fluorescent dye for detection of GAGs

Most of the commercially available equipments for microchip electrophoresis are designed for detection of nucleic acids, which form fluorescent complexes by intercalation with a fluorescent dye in buffer. Detection is usually performed with a semiconductor laser as a light source at 518 nm. In the present study, we examined to detect native GAGs using a commercially available microchip electrophoresis apparatus in the presence of a dye employed for detection of nucleic acids. Fig. 1 shows the list of the dyes examined in this study.

When HA (1 mg/ml) was analyzed in the presence of ethidium bromide (b) as a dye reagent at 0.001% concentration in 0.1 M tris-acetate buffer (pH 7.5) containing 1% polyethyleneglycol, an intense peak of HA was observed. Propidium iodide (c) also showed good results. But other reagents (a), (d), and (e) did not show obvious HA peak (date not shown). Although ethidium bromide (b) and propidium iodide (c) gave similar fluorescence intensity, we selected ethidium bromide (b) in this study due to its low running cost.

We examined the optimum concentration of ethidium bromide, and found that higher concentrations than 0.001% caused increase of background fluorescence. Lower concentrations than 0.001% of ethidium bromide decreased peak response (data not shown).

3.2. Analysis of hyaluronic acid (HA)

HA is widely used for medical use such as treatment of arthritis or an ingredient of eye drops. Although we reported the analysis of native HA preparations and HA oligomers using capillary electrophoresis with photometric detection, the analysis requires ca. 1 h [14,22–24]. In contrast, the present method using microchip electrophoresis is completed within a few minutes. Fig. 2A shows the results on the analysis of three commercial HA preparations from different sources in the buffer containing ethidium bromide. In Fig. 2B, the same HA preparations were analyzed using conventional cellulose acetate membrane electrophoresis.

Preparation (a) is derived from pig skin, (b) from rooster comb and (c) from *S. zooepidemicus*, respectively. The low-molecular mass preparation (ca. 40,000–60,000 Da) derived from pig skin showed a small peak at ca. 110 s (Fig. 2A(a)). In contrast, an HA preparation (ca. 800,000 Da) derived from rooster comb showed a peak at 125 s along with a small peak at 95 s (Fig. 2A(b)). A high-molecular mass preparation (higher than 1,000,000 Da) derived from *S. zooepidemicus* also showed two peaks at 95 and 125 s. However, the peak intensity at 95 s was more abundant than that observed for the

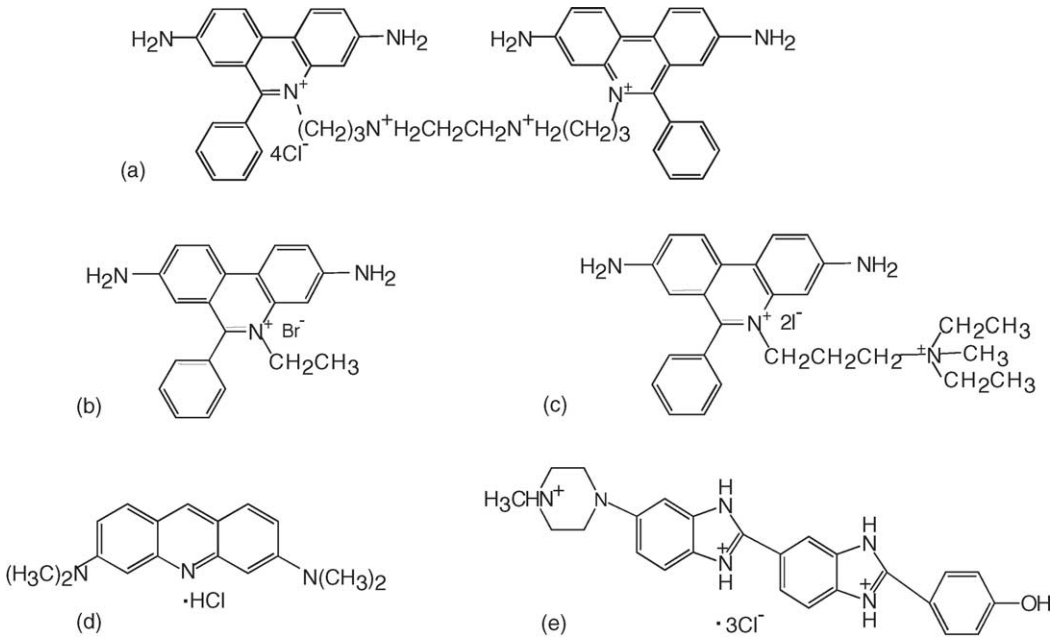

Fig. 1. Fluorescent dyes examined for detection of GAGs: (a) ethidium homodimer-1 (Ex 528 nm, Em 617 nm); (b) ethidium bromide (Ex 518 nm, Em 605 nm); (c) propidium iodide (Ex 535 nm, Em 617 nm); (d) acridine orange (Ex 500 nm, Em 526 nm); (e) Hoechst 33258 (Ex 352 nm, Em 461 nm).

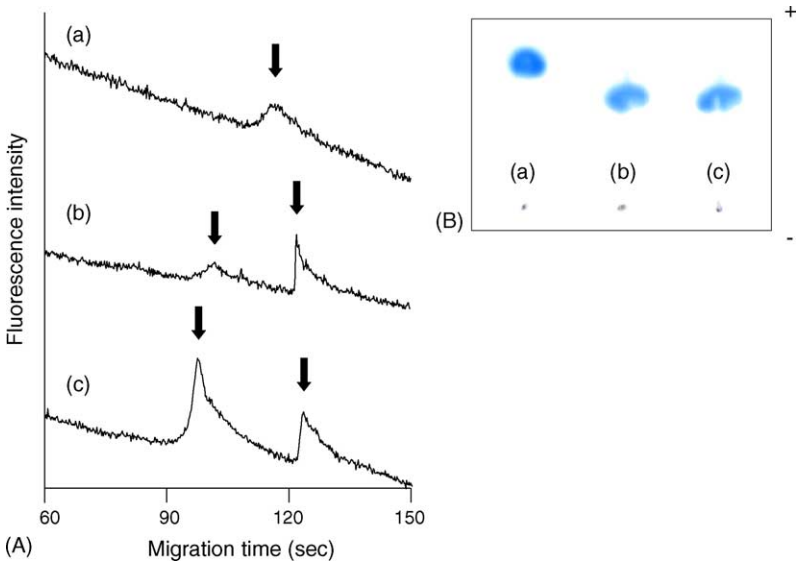

Fig. 2. Analysis of hyaluronic acid preparations. (A) Microchip electrophoresis: (a) low-molecular-mass hyaluronic acid (average molecular mass, 40,000–60,000 Da) derived from pig skin, (b) hyaluronic acid (rooster comb) having molecular size of 800,000 Da, and (c) hyaluronic acid (*S. zooepidemicus*) having molecular size of higher than 1,000,000 Da. Analytical conditions: buffer; 0.1 M tris-acetate/1% polyethyleneglycol 70,000/0.001% ethidium bromide (pH 7.5); applied voltage, for sample injection: 300 V; for analysis: 750 V. Each sample was analyzed at 1 mg/ml solution. Arrows indicate the peak of hyaluronic acid. (B) Analysis by cellulose acetate membrane electrophoresis. About 1.0 μg portion of each sample was applied on the membrane.

analysis of the HA preparation derived from rooster comb. In contrast, these preparations showed single spot when analysed by cellulose acetate membrane electrophoresis, and the low-molecular mass preparation derived from pig skin migrated faster than other two preparations, probably because of low viscosity (Fig. 2B).

Scott et al. reported several important and suggestive papers on the mechanism of fluorescent detection of HA using an intercalator reagent [25,26]. Computer simulation and energy calculation indicate that HA molecules form twofold helix. These observations indicate that the intercalator molecules occupy the positions in hydrophobic cavity of the helix and show enhancement of fluorescence. In the present study, we found that ethidium homodimer-1 (Fig. 1(a)) did not show enhancement of fluorescence. But ethidium bromide and propidium iodide (Fig. 1(b) and (c)) enhance fluorescence upon addition to an aqueous solution of HA. These results indicated that the size of the reagent molecules, namely single 9-phenyl-phenanthridinium structure, is important and just fits the hydrophobic cavity of HA. In contrast, acridine orange (Fig. 1(d)) and Hoechst 33258 (Fig. 1(e)) did not show increase in fluorescence. These reagents do not have alkyl groups in their molecules. The alkyl groups of ethidium bromide also seem to be important for enhancement of fluorescence.

In the previous paper, we reported that HA molecules having higher molecular masses than those derived from pig skin are preferable for complex formation with poly-L-lysine [27]. Low intensity of the HA peak derived from pig skin (Fig. 2A(a)) is well understood from this observation. Low-molecular mass HA preparation (i.e. HA preparation having low-viscosity) occasionally shows single filaments, but HA of high molecular-masses forms networks and shows an irregular honeycomb structure under observation using rotary shadowing and electron microscopy [26]. HA molecules are composed of repeating (-4GlcAβ1-3-GlcNAcβ1-) units, and are highly heterogeneous in molecular mass distributions. Therefore, HA molecules of low-molecular masses derived from pig skin showed only a small peak at 110 s. In contrast, HA molecules having larger molecular masses contain a network form as well as single filaments in solution, and showed two peaks as shown in Fig. 2A(b) and 2A(c). A large peak at 95 s as well as a smaller one at 125 s suggested that both HA preparations derived from rooster comb and *S. zooepidemicus* have two different molecular forms as reported by Scott [26]. Although we have no approaches to examine solution conformation of HA molecules, these observations will be useful for understanding HA matrix in biological tissues.

3.3. Detection limit and calibration curves

We examined lower detection limit of HA preparations. The results are shown in Fig. 3.

Detection limit of HA derived from pig skin was 1.0 mg/ml with signal to noise ratio of ca. 3 (Fig. 3A(a)). High molecular mass HA preparations derived from rooster comb and *S. zooepidemicus* showed higher sensitivities and were detected at the concentration of 0.1 mg/ml with signal to noise ratio of ca. 3 and 5, respectively. Sensitivities of the present method were comparable with those of conventional cellulose acetate membrane electrophoresis. But we will be able to improve the sensitivity by customizing the system for glycosaminoglycans.

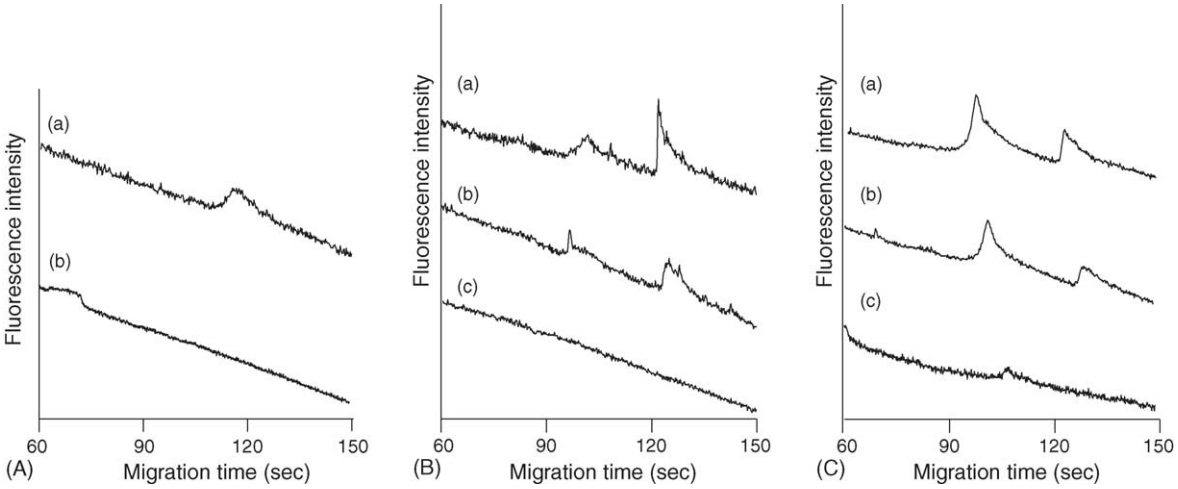

Fig. 3. Lower detection limit of hyaluronic acid. (A) Hyaluronic acid derived from pig skin at (a) 1.0 mg/ml and (b) 0.5 mg/ml. (B) Hyaluronic acid derived from rooster comb at (a) 1.0 mg/ml, (b) 0.1 mg/ml, and (c) 0.05 mg/ml. (C) Hyaluronic acid derived from *S. zooepidemicus* at (a) 1.0 mg/ml, (b) 0.1 mg/ml, and (c) 0.05 mg/ml. Analytical conditions were the same as in Fig. 2.

We observed narrow range of calibration curve (0.1–1.0 mg/ml for HA, data not shown). This narrow range of calibration curve is mainly due to the background fluorescence of ethidium bromide. High level of background fluorescence of ethidium bromide results failure in detection of GAGs at low concentrations. However, it should be noted that the present method using microchip electrophoresis is ca. 100 times more rapid than conventional cellulose acetate membrane electrophoresis, and is completed within 150 s.

3.4. Analysis of sulfated glycosaminoglycan samples

In the analysis of HA by capillary electrophoresis, polyethyleneglycol was used as molecular-sieving material [28]. Fig. 4 shows effect of polyethyleneglycol in the running buffer on the peak response when heparin (HP) and chondroitin sulfate A (CSA) were used as samples.

In the presence of polyethyleneglycol, HP (Fig. 4A(a)) and CSA (Fig. 4B(a)) were observed at 65 and 70 s, respectively. However, fluorescence intensities of these peaks were smaller than those in the absence of polyethyleneglycol (HP, Fig. 4A(b), CSA, Fig. 4B(b)). In microchip electrophoresis, sample solutions were introduced by electrokinetic method. This was probably due to difference in injection efficiency. Therefore, sulfated glycosaminoglycans were analyzed in the absence of polyethyleneglycol.

In the similar manner, we examined lower detection limit in the analysis of some sulfated glycosaminoglycans. Migration times and detection limits of all typical GAGs are summarized in Table 1.

Heparin (HP) was observed at ca. 60 s, and a solution at 0.25 mg/ml was detected with signal to noise ratio of ca. 3. A preparation of low-molecular HP was also observed at ca. 60 s. Sensitivity was not so high as that observed for HP and detection limit was 1.0 mg/ml with signal to noise ratio of ca. 5. This observation that low molecular heparin shows lower sensitivity than that of high molecular one is well correlated with that observed in the analysis of HA. Heparan sulfate (HS) was observed at 80 s. The slow migration was due to the lower content of sulfate groups than HP, and broad peak indicated that carbohydrate chains of HS were highly heterogeneous (date not shown). Preparations of chondroitin sufate A (CSA), chondroitin sulfate C (CSC), and dermatan sulfate (DS) were also analyzed in the similar manner and observed at ca. 65 s. Detection limits of CSA, CSC and DS were 0.1, 0.1 and 0.2 mg/ml, respectively. The present microchip device requires 10 μl of sample solution for analysis. Therefore,

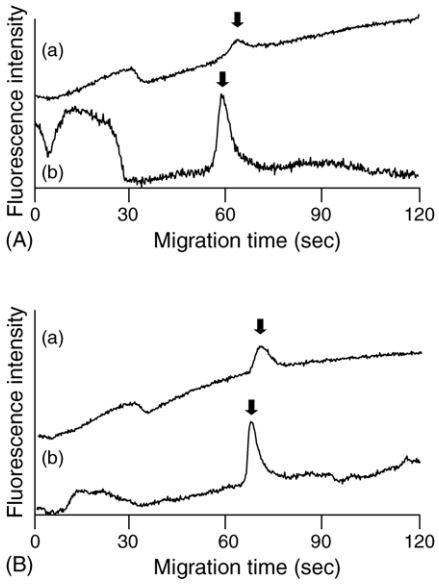

Fig. 4. Effect of polyethyleneglycol in the running buffer on the analysis of sulfated GAGs: heparin (A) and chondroitin sulfate A (B) were analyzed in the presence (a) or absence (b) of polyethyleneglycol. Each sample was analyzed at 1 mg/ml. Arrows indicate the peak of heparin or chondroitin sulfate A. Analytical conditions were the same as in Fig. 2.

Table 1
Lower-detection limit and migration times of GAG samples

Glycosaminoglycan	Origin	Migration time (s)	Detection limit (μg/10 μl)
Hyaluronic acid	Pig skin	110	10
Hyaluronic acid	Rooster comb	95, 125	1.0
Hyaluronic acid	*S. zooepidemicus*	95, 125	1.0
Heparin	Porcine intestine	60	2.5
Heparin (low-molecular-weight)	Porcine intestine	60	10
Heparan sulfate	Bovine kidney	80	5.0
Chondroitin sulfate A	Whale cartilage	65	1.0
Chondroitin sulfate A	Shark cartilage	65	1.0
Dermatan sulfate	Pig skin	65	2.0

sensitivity was microgram level, which was comparable with that using cellulose acetate membrane [29].

3.5. Reproducibility

Run-to-run reproducibility of migration time is evaluated using GAGs preparations. The standard errors showed less than 1.1% ($n = 5$) in the analysis of all GAGs preparation.

3.6. Analysis of acidic polysaccharides

We applied the present technique to the analysis of other acidic macromolecules such as colominic acid, dextran sulfate, fucoidan, and alginic acid. Colominic acid, a polysaccharide composed from *N*-acetylneuraminic acid through α2–8 linkages, has various important biological functions, and the relationship between the chain length and its biological activity has attracted attentions [30]. Dextran sulfate is used for treatment of hyperlipidemia. Fucoidan is a polymer of fucose, and highly sulfated. Fucoidan has attracted attention as an additive for health food in Japan [31]. Alginic acid, composed from D-mannuronic acid and L-gluronic acid, is available as sodium salt, and obtained from brown algae. An aqueous solution of alginic acid has been used for protection of mucus layer of digestive organs. Fig. 5 shows the results on the analysis of these polysaccharides.

Colominic acid (a), dextran sulfate (b), and fucoidan (c) were observed at 70, 60, and 65 s, respectively. However, sodium alginate could not be detected (date not shown). These results indicated that fluorescence development by the intercalator was closely correlated with structures of polysaccharides, because colominic acid has been reported to form single helix structure [28]. Such structure is required for fluorescent formation, although the fluorescence intensity is not so high as those observed in double stranded structures of nucleic

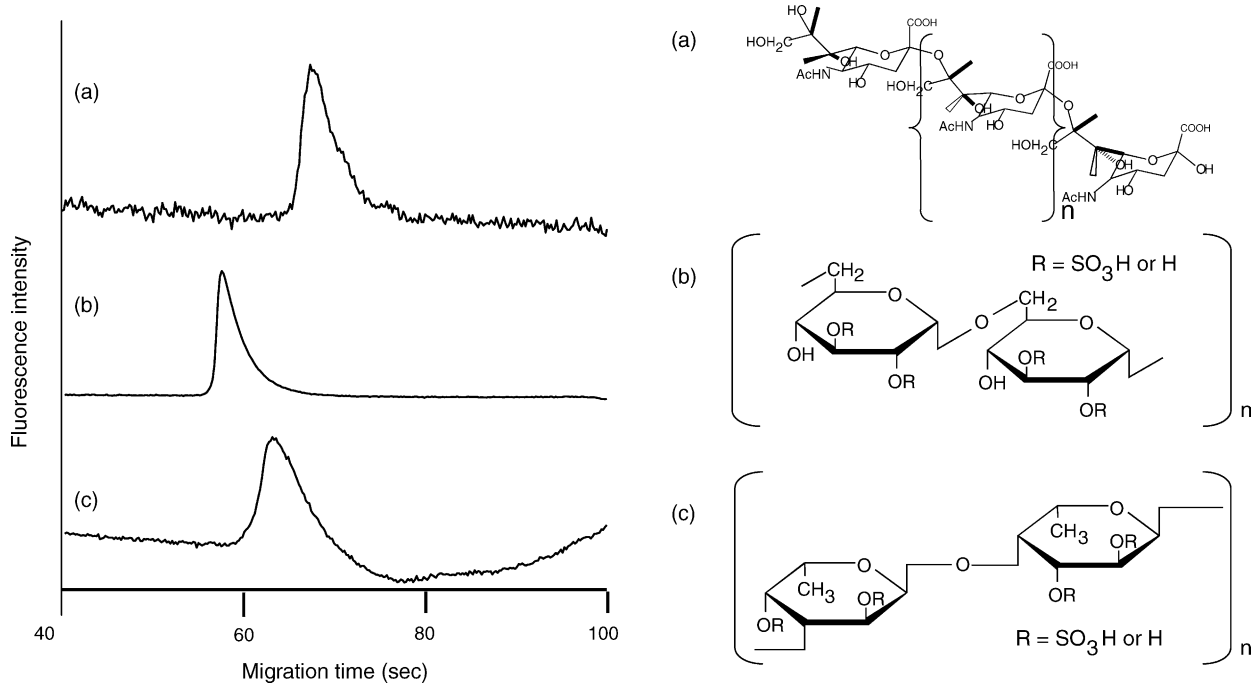

Fig. 5. Analysis of acidic polysaccharides: (a) colominic acid, (b) dextran sulfate, (c) fucoidan. Analytical conditions were the same as in Fig. 4(b). Each sample was analyzed at 1 mg/ml solution.

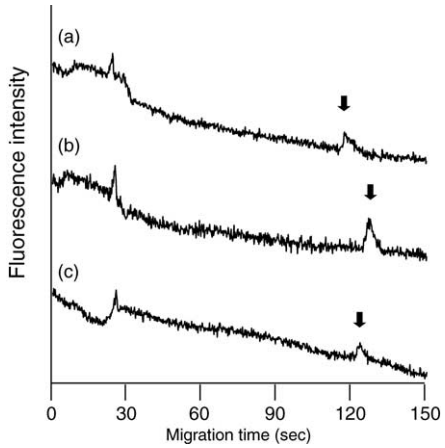

Fig. 6. Analysis of pharmaceutical preparations of hyaluronic acid: (a) and (b) preparations for treatment of arthritis; (c), preparation for treatment of ophthalmic surgery. Analytical conditions were the same as in Fig. 2. Arrows indicate the peak of hyaluronic acid. Concentrations of hyaluronic acid in all preparations were 10 mg/ml according to the manufacturers' data sheets.

acids. Detection limits of these acidic polysaccharides were 1.0–2.0 μg as injected amount.

3.7. Application to the analysis of pharmaceutical GAG preparations

We applied the present method to the analysis of pharmaceutical preparations of GAGs as shown in Fig. 6.

HA preparations (a) and (b) for treatment of arthritis showed two peaks at ca. 30 and 120 s. The preparation (c) for ophthalmic surgery, also gave two peaks at the similar migration times. The peak observed at ca. 30 s could not be identified but presumably derived from additives in the preparations. These preparations showed only one peak at ca. 120 s, but another peak observed in the analysis of HA preparations derived from *S. zooepidemicus*, was not observed. As shown in Fig. 2, HA derived from *S. zooepidemicus* clearly showed two peaks, and the peak observed at 95 s was more abundant than that observed in rooster comb. If the HAs analyzed in Fig. 6 are derived from *S. zooepidemicus*, the peak observed at 95 s must be observed. In addition, this peak was disappeared by digestion with hyaluronidase (data not shown). Therefore, we conclude that these HA preparations are derived from rooster comb. We also analyzed some commercial pharmaceutical heparin preparations (Fig. 7).

Preparations (a) and (b) are the products from pig intestine, and the preparation (c) is a low-molecular-weight HP preparation. Preparations (a) and (b) gave incompletely resolved two peaks at 60 and 65 s, but the low-molecular-weight preparation showed a peak at 60 s. However, in the analysis by cellulose acetate membrane electrophoresis, preparations (a) and (b) were observed as single spot at the same position with that of a standard HP sample. These results indicate that HP preparations (a) and (b) have higher heterogeneities than low-molecular-weight heparin preparations.

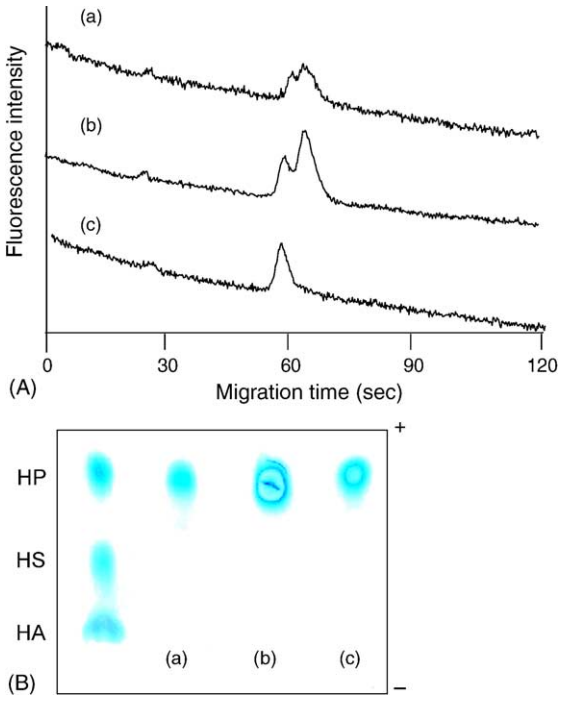

Fig. 7. Analysis of pharmaceutical preparations of heparin: (A) microchip electrophoresis; (B) cellulose acetate membrane electrophoresis: (a) and (b) are heparin preparations from pig intestine, and (c) is the low-molecular-weight heparin preparation. Analytical conditions were the same as in Fig. 4(b). All preparations are 1000 units/ml. HP, heparin; HS, heparan sulfate; HA, hyaluronic acid.

3.8. Application to the analysis of GAGs derived from HeLa cells

As another application of the present method, we attempted to analyze GAGs in cultured HeLa cells (Fig. 8).

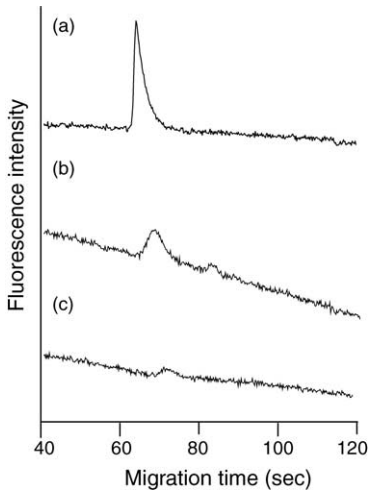

Fig. 8. Analysis of GAGs derived from HeLa cells: (a) standard sample of chondroitin sulfate A derived from whale cartilage; (b) GAGs derived from HeLa cells; (c) GAGs derived from HeLa cells after digestion with chondroitinase ABC. Analytical conditions were the same as in Fig. 4(b).

Crude GAGs obtained from HeLa cells gave a small peak at 70 s (Fig. 8(b)), which showed the similar migration time with that of standard chondroitin sulfate. Digestion with chondroitinase ABC resulted disappearance of the peak (Fig. 8(c)). Furthermore, digestion products of chondroitin sulfates by chondroitinase ABC was compared with standard unsaturated disaccharides according to the method reported previously [32], and we could confirm peaks due to ΔDi-diS, ΔDi-6S, and ΔDi-4S (date not shown). These results indicate that chondroitin sufates are abundantly present in cultured HeLa cells. Analysis of GAGs in biological samples has been a time-consuming and laborious work. In the present study, we could propose an easy method for examining the presence of GAGs.

4. Conclusion

In the present study, we applied microchip electrophoresis to the analysis of native GAGs with in situ fluorescent detection and found that intercalating reagents for gel electrophoresis of nucleic acids were useful for detection of GAGs. By using buffer solution containing ethidium bromide, GAGs could be directly analyzed within 150 s. This approach provided utmost 100-fold rapid analysis compared with the conventional electrophoresis technique using cellulose acetate membrane. Lower detection limit was microgram level. However, we could not obtain good linearity for calibration curves, because the present apparatus was customized for the analysis of nucleic acids.

We applied the present method to the analysis of some acidic polysaccharides such as colominic acid, dextran sulfate, and fucoidan. All the polysaccharides except for alginic acid were detected within 80 s with the similar sensitivity to those observed for the GAGs analysis. The results indicated that fluorescence formation by the intercalating reagent is specifically correlated with structures of the acidic polysaccharides.

We analyzed GAGs in some pharmaceutical GAG preparations and GAGs derived from HeLa cells, and showed that the rapidness was quite useful as an alternate using conventional electrophoresis technique.

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